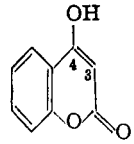
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF WISCONSIN]

Studies on 4-Hydroxycoumarin. VII. Reactions of 4-Hydroxycoumarin with Cationoid Reagents[1]

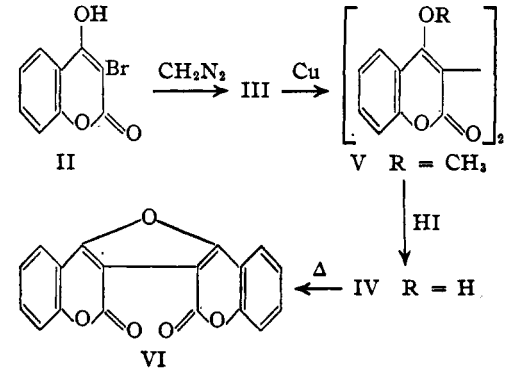
BY CHARLES F. HUEBNER WITH KARL PAUL LINK

The system involving carbons 3 and 4 of 4-hydroxycoumarin (I) is in the main responsible for the chemical reactivity of the molecule. This grouping as a part of the 4-hydroxycoumarin molecule has been shown to be essential for anticoagulant activity.[2] I showed a 98–100% enol content in ethanol at 7° as determined by the direct and indirect method of Kurt Meyer.[3] Completeness of enolization is also indicated by the acidity of I ($Ka = 2.3 \times 10^{-6}$). The present paper describes the reactions of I with certain cationoid reagents with which carbon 3 reacts. Previously, tetronic acid has been shown to exhibit similar reactions.[4]



I

3-Bromo-4-hydroxycoumarin (II) is prepared by bromination of I in chloroform. In contrast to typical bromo phenols, II was reduced with hydriodic acid to yield I. This shows that the bromine could not have been on the aromatic nucleus and must be on carbon 3. 3-Bromo-4-methoxycoumarin (III) was used in an attempted synthesis of 3,3′-bis-(4-hydroxycoumarin) (IV), the lower homolog of the anticoagulant, 3,3′-methylenebis - (4 - hydroxycoumarin). Diazomethane was used to methylate II. The Ullmann reaction on III yields a crude 3,3′-bis-(4-hydroxycoumarin) dimethyl ether (V). This product upon demethylation with hydriodic acid in acetic acid yields a mixture of a neutral and alkali-soluble fraction. On sublimation and recrystallization of the alkali-soluble fraction from acetic acid, complete conversion to the neutral compound occurs. The analysis was in agreement with an anhydro product derived from IV and it is designated 3,3′-4,4′-epoxydicoumarin (VI). On acidification of the alkali soluble fraction part of it is converted to the anhydro product. It then becomes insoluble in alkali. Each time this process is repeated a portion of IV is cyclized to VI. Thus, as indicated by analysis of the product obtained by acidifying such a base soluble fraction, 3,3′-bis-(4-hydroxycoumarin) can be obtained only in a mixture with its anhydro compound.



Nitration of I to the 3-nitro derivative is accomplished by the use of one equivalent of nitric acid in acetic acid. On alkaline oxidation 3-nitro-4-hydroxycoumarin (VII) yields salicylic acid demonstrating that the nitro group occupies position 3. Hydrogenation of VII gives the corresponding amino compound (VIII). As a characteristic derivative of VIII, the N acetate was prepared by the Chattaway technique.[5] On diazotization VIII yields 3,4-diazocoumarin oxide (IX). The reduction of 3-nitro-4-hydroxycoumarin (VII) by hydriodic acid produces β-amino-*o*-hydroxyacetophenone (X), decarboxylation as well as reduction occurring. This amino ketone is stable only as a hydrochloride. The free base condenses immediately to yield 2,5-di-(*o*-hydroxyphenyl)-dihydropyrazine (XI). The analogous reaction of β-aminoacetophenone has been described by Gabriel.[6] Five per cent. sodium hydroxide at room temperature decarboxylates (VII) to β-nitro-*o*-hydroxyacetophenone (XII). Treatment of XII with acetic anhydride and pyridine gives a yellow anhydromonoacetate. This compound is probably 2-methyl-3-nitro-benzo-γ-pyrone (XIII).

Under more drastic nitration conditions a second nitro group is introduced into the 6 position of 4-hydroxycoumarin. This product is soluble in the nitrating mixture and moderately soluble in water. The best yields of 3,6-dinitro-4-hydroxycoumarin (XIV) are obtained when an optimum amount of water is used to precipitate the compound from its acid solution. Hence yields are generally low because of this difficulty. Simultaneous oxidation to produce nitrated salicylic acids also lowers the yield.

Sulfonation of I is effected with fuming sulfuric

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station and supported since July, 1940, through special grants from the Graduate Research Committee, Office of Dean E. B. Fred, and the Wisconsin Alumni Research Foundation.

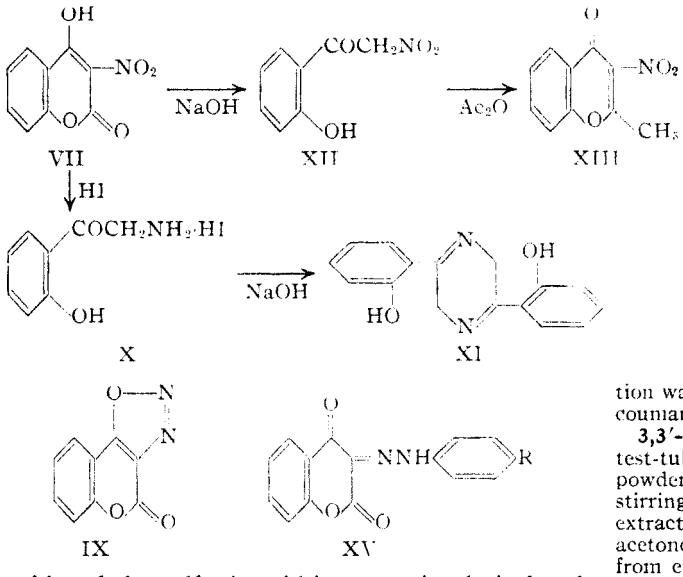
(2) Overman, *et al.*, *J. Biol. Chem.*, **153**, 5 (1944).

(3) Meyer, *Ber.*, **44**, 2718 (1911).

(4) Wolff, *Ann.*, **291**, 226 (1896); **312**, 119 (1900).

(5) Chattaway, *J. Chem. Soc.*, 2495 (1931).

(6) Gabriel, *Ber.*, **41**, 1127 (1908).



acid and the sulfonic acid is conveniently isolated as a glycine salt. The sulfonate group is removed by formaldehyde and 3,3′-methylenebis-(4-hydroxycoumarin) is formed. For this reason, the 3 position is assigned to the sulfonate group.

4-Hydroxycoumarin couples with diazotized amines in sodium carbonate solution. Soon after coupling, an insoluble product appears without acidification. For this reason the hydrazone structure is assigned to these compounds (XV). Proof that the phenylhydrazine residue occupies position 3 resides in the fact that these compounds may be reduced by hydriodic acid to produce the previously described 3-amino-4-hydroxycoumarin (VIII).

The authors wish to thank Dr. William Sullivan and Mr. Lloyd Graf for the C and H determinations and Mr. Saul Roseman for assistance with some of the experimental work.

Experimental

Enol Determination on 4-Hydroxycoumarin.—The determination was carried out as described by Meyer.[3] Measurements made on acetoacetic ester gave correct values for per cent. enol. Measurements were made in ethanol at 7°.

Control on Acetoacetic Ester.—Sample wt. 1.030 g.; ml. bromine soln. (0.0750 *M*), 8.40; enol (direct), 7.8%; ml thiosulfate (0.1075 *N*), 11.60; enol (indirect), 7.8%.

4-Hydroxycoumarin.—Sample wt., 0.3360 g.; ml. bromine soln., 28.30; enol (direct), 102%; ml. thiosulfate, 38.00; enol (indirect), 98%; sample wt., 0.3117 g ; ml. thiosulfate, 35.80; enol, 100%.

3-Bromo-4-hydroxycoumarin (II).—To a well-stirred suspension of 29 g. of I in 150 ml. of chloroform cooled in ice there was added over a period of one hour, one molecular equivalent plus a 10% excess of 1.5 molar bromine in chloroform. After standing for another hour, the slurry of the bromo compound was filtered, pressed dry and recrystallized from ethanol, yield 27 g., m. p. 190–192°.

Anal. Calcd. for $C_9H_5O_3Br$: C, 44.8; H, 2.1; neutral equivalent, 241. Found: C, 44.8; H, 2.2; neutral equivalent, 242.

3-Bromo-4-methoxycoumarin (III).—The diazomethane prepared from 20 g. of nitrosomethylurea was distilled into a suspension of 22 g. of II in 150 ml. of dry ether cooled to 5°. After the reaction had been completed, two kinds of crystals were noted in the mixture. A mechanical separation of a few of the crystals showed that one type melted at 75–80° and the other at 140–142°. Fractionation through the use of solvents failed. The ether solution was evaporated to half its volume and filtered. The resulting mixture was distillated at 5 mm.; the lower melting compound distilled at 185–187°, yield 12 g. After recrystallization from ethanol the m. p. was 85–88°.

Anal. Calcd. for $C_9H_4O_2Br(OCH_3)$: OCH_3, 12.2. Found: OCH_3, 12.3.

Conversion of 3-Bromo-4-hydroxycoumarin (II) to I.—II or III (400 mg.) was refluxed for fifteen min. in a mixture of 5 ml. of acetic acid and 2 ml. of hydriodic acid (58%). The solution was poured into ice water and 200 mg. of 4-hydroxycoumarin (I) crystallized, m. p. 210–212°.

3,3′-4,4′-Epoxydicoumarin.—III (5 g.) was melted in a test-tube and heated to 210°. A double weight of copper powder was added and the heating was continued with stirring for one-half an hour. The melt was pulverized and extracted with acetone in a Soxhlet for three days. The acetone was removed and the residue recrystallized once from ethanol and once from benzene, yield 1.2 g., m. p. 215–218°. The crude dimethyl ether (V) was demethylated by refluxing it for fifteen minutes in 58% aqueous hydriodic acid and acetic acid (1:2). The crude product was filtered and sublimed at 250° and 0.05 mm. The sublimate was recrystallized from acetic acid, m. p. 310–320°.

Anal. Calcd. for $C_{19}H_{10}O_5$: C, 71.0; H, 2.6. Found: C, 70.8; H, 2.9.

When the crude demethylated mixture was extracted with alkali, the alkaline solution acidified and the precipitated product subjected to the same treatment three more times, a crude preparation of 3,3′-bis-(4-hydroxycoumarin) (IV) was obtained. Based on the C and H analysis it contained 25% of the anhydro compound. Each time a basic solution of 3,3′-bis-(4-hydroxycoumarin) is acidified, one-fourth of the alkali-soluble compound is converted to the neutral anhydro compound.

3-Nitro-4-hydroxycoumarin (VII).—To a suspension of 20.5 g. of I in 50 ml. of acetic acid was added 9.2 ml. of nitric acid dissolved in 10 ml. of acetic acid. The mixture was placed on a steam-bath and the temperature raised to 80° at which point the nitration suddenly began. The flask was then cooled in ice to keep the reaction under control. The crude product was filtered and recrystallized once from ethanol, yield 14 g., m. p. 177° (dec.).

Anal. Calcd. for $C_9H_5O_5N$: C, 52.1; H, 2.4; N, 6.8; neutral equivalent, 207. Found: C, 52.3; H, 2.9; N, 6.7; neutral equivalent, 208.

Oxidation of 3-Nitro-4-hydroxycoumarin (VII) to Salicylic Acid.—VII (1 g.) and 1.5 g. of potassium hydroxide were dissolved in 50 ml. of water. An aqueous solution of 2.36 g. of potassium permanganate was added over a period of one-half an hour. The precipitate of manganese dioxide was reduced by sulfur dioxide. The excess sulfur dioxide was then removed by washing with air. The solution was made basic with a potassium hydroxide solution until the white manganous hydroxide precipitated. This was centrifuged off and the supernatant liquid acidified and extracted with ether. Upon evaporation of the ether, 100 mg. of salicylic acid remained. It was recrystallized from water, m. p. 158–160°.

3-Amino-4-hydroxycoumarin Hydrochloride (VIII) and 3-Amino-4-hydroxycoumarin N-Acetate.—VII (13 g.) was suspended in 500 ml. of methanol to which had been added 22 ml. of 3% hydrogen chloride in methanol. The catalyst (palladium on carbon) was added and the hydrogenation (at one atmosphere) begun. The reaction was complete after two hours and partial cry allization of the amine hydrochloride occurred. The s aid was filtered and the separation of the amine hydrochlo ide from the catalyst was accomplished by extraction with a mixture of hot

TABLE I

2,3,4-TRIKETOCHROMANE-3-*p*-SUBSTITUTED PHENYLHYDRAZONES

p-Substituent	M. p., °C.	Yield, %	Recrystallized from	Formula	Nitrogen, % Calcd.	Nitrogen, % Found	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
-Hydrogen	178–179	91	HOAc	$C_{15}H_{10}N_2O_3$	10.6	10.4	67.9	67.9	3.4	3.7
-Methyl	188–190	88	HOAc	$C_{16}H_{12}N_2O_3$	10.0	10.3	57.9	58.0	2.9	3.4
-Nitro	265	75	$C_6H_5NO_2$	$C_{15}H_9N_3O_5$	13.3	13.4				
-Sulfonamide	298	50	Phenol	$C_{15}H_{11}N_3O_6S$	12.0	12.0				
-Sodium sulfonate	Above 350	10	Water	$C_{15}H_9N_2O_6SNa$	8.4	8.2				

ethanol and concentrated hydrochloric acid (4:1). The combined filtrates were then concentrated to about 20 ml. and the crude product filtered. The yield was 9 g., m. p. 210–215°. It was not possible to realize satisfactory analyses on the hydrochloride, so the monoacetate was prepared as a characteristic derivative for analysis.

After suspension of the amine hydrochloride (0.6 g.) in 20 ml. of ice-water, 1 ml. of acetic anhydride and an excess of sodium acetate were added and the whole shaken until the crystalline monoacetate appeared. Upon recrystallization from ethanol the product melted at 222–224°, yield, 0.4 g. That the product was a N-acetyl compound was shown by its solubility in sodium bicarbonate solution and its recovery by acidification of the basic solution.

Anal. Calcd. for $C_{11}H_9O_4N$: C, 60.2; H, 4.1; N, 6.4. Found: C, 59.9; H, 4.1; N, 6.4.

3,4-Diazocoumarin Oxide (IX).—To a suspension of VIII (2 g.) in ice-water, 1.5 molar equivalents of 1 *N* hydrochloric acid and 1 molar equivalent of sodium nitrite solution were added in succession with shaking. The mixture was allowed to stand for one-half an hour in the icebox. The product crystallized from ethanol in long white needles, m. p. 156–157°, yield 1.2 g.

Anal. Calcd. for $C_9H_4O_3N_2$: N, 14.9. Found: N, 15.0.

β-Amino-*o*-hydroxyacetophenone Hydrochloride (X).—Ten grams of VII was refluxed in a mixture of 10 ml. of hydriodic acid (58%) and 20 ml. of acetic acid for fifteen minutes. The iodine produced during the reaction was reduced with hypophosphorous acid. The solution was concentrated under reduced pressure to a small volume, whereupon crystallization occurred. The product was filtered and recrystallized twice from concentrated hydrochloric acid, m. p. 229–230°, yield 6 g.

Anal. Calcd. for $C_8H_9O_2N \cdot HCl$: N, 7.5. Found: N, 7.4.

2,5-Di-(*o*-hydroxyphenyl)-dihydropyrazine (XI).—X (3.2 g.) was dissolved in water and one equivalent of 0.6 *N* potassium hydroxide was added. An immediate gelatinous pink precipitate appeared. The product was filtered and recrystallized twice from dioxane, m. p. 262–264°, yield 1.3 g. XI is yellow in color, insoluble in base and when the crystals are placed in concentrated hydrochloric acid, red hydrochloride crystals form in place of the original yellow base.

Anal. Calcd. for $C_{16}H_{14}O_2N_2$: C, 72.1; H, 5.3; N, 10.5. Found: C, 72.4; H, 5.1; N, 10.6.

β-Nitro-*o*-hydroxyacetophenone (XII).—VII (4.2 g.) was dissolved in 150 ml. of 5% sodium hydroxide and allowed to stand at 20° for twenty-four hours. Upon acidification the crude decarboxylation product was realized. It was recrystallized from ethanol, yield 2.9 g., m. p. 106–107°.

Anal. Calcd. for $C_8H_7O_4N$: C, 53.0; H, 3.9; N, 7.7. Found: C, 53.2; H, 3.9; N, 7.7.

1-Methyl-2-nitro-benzo-γ-pyrone (XIII).—On acetylation of XII (3 g.) at room temperature in 5 ml. of pyridine and 5 ml. of acetic anhydride, yellow needles separated from the mixture. They were filtered and recrystallized from ethanol, m. p. 181–182°, yield 0.6 g.

Anal. Calcd. for $C_{10}H_7O_4N$: C, 58.5; H, 3.4; N, 6.8; molecular weight, 205. Found: C, 58.6; H, 3.5; N, 6.9; molecular weight, 222 (Rast).

3,6-Dinitro-4-hydroxycoumarin (XIV).—I (10 g.) was dissolved in 20 ml. of sulfuric acid and 20 ml. of fuming nitric acid was added. As the reaction proceeded the heat evolved was dissipated by cooling the flask in ice-water. The mixture was poured into 100 g. of ice and the yellow product that precipitated was filtered and recrystallized from a mixture of benzene and ethanol (9:1), yield 5.6 g., m. p. 185° (dec.). Another recrystallization raised the m. p. to 188°. The compound exhibits a slight yellow color.

Anal. Calcd. for $C_9H_5O_5N_2$: C, 42.8; H, 1.6; N, 11.1. Found: C, 42.8; H, 1.7; N, 11.2.

3,6-Dinitro-4-methoxycoumarin.—The methyl ether was prepared by the usual diazomethane technique. It was recrystallized from acetic acid, m. p. 208–210°.

Anal. Calcd. for $C_9H_4O_4N_2(OCH_3)$: OCH_3, 11.7. Found: OCH_3, 11.5.

Oxidation of 3,6-Dinitro-4-hydroxycoumarin (XIV) to 5-Nitrosalicylic Acid.—XIV (150 mg.) was oxidized in a basic solution with 285 mg. of potassium permanganate and the acidic fraction was isolated as described for the oxidation of 3-nitro-4-hydroxycoumarin. 5-Nitrosalicylic acid was isolated, m. p. 225–227°. The methyl ester was prepared with diazomethane, m. p. 99–100°.

4-Hydroxycoumarin-3-sulfonic Acid (Glycine and Sodium Salt).—Ten grams of I was added with stirring to 20 ml. of fuming sulfuric acid. After standing twelve hours, the brown sirup was poured into 500 ml. of ice-water and 8 g. of glycine was added. After two days the glycine salt (6 g.) was filtered. It was recrystallized from water, m. p. 225–230°.

Anal. Calcd. for $C_{11}H_{11}O_8NS$: C, 41.6; H, 3.5; N, 4.4. Found: C, 42.0; H, 3.6; N, 4.2.

A sample of the glycine salt was dissolved in the minimum of hot water and five times its weight of sodium acetate was added. Upon cooling the sodium salt of the sulfonic acid crystallized. After two recrystallizations from water, the ash content was determined. The sample was dried *in vacuo* at room temperature.

Anal. Calcd. for $C_9H_5O_6SNa \cdot 2H_2O$: Na, 8.6. Found: Na, 8.5.

A hot solution of the sodium or glycine salt in water when treated with a formaldehyde solution, immediately gave a precipitate of 3,3′-methylenebis-(4-hydroxycoumarin).

2,3,4-Triketochromane-3-*p*-substituted Phenylhydrazones (XV).—The various 3-phenylhydrazino compounds were all prepared in essentially the same manner. Ten grams of I was dissolved in a solution of 15 g. of sodium carbonate (anhydrous) in 250 ml. of water. To this solution cooled to 5°, one equivalent plus a 5% excess of the diazotized amine was added with stirring. After one hour at 5°, complete formation of the base insoluble coupled product had occurred. The solution was acidified with acetic acid, the product filtered, washed and recrystallized from an appropriate solvent. An exception to this scheme of isolation was in the method used in the case of the coupled product with diazotized sulfanilic acid. Since the coupled product is extremely soluble in water, it was isolated as the sodium salt by saturating the solution with sodium acetate. All of these products are yellow. Table I summarizes the properties of these compounds.

3-Amino-4-hydroxycoumarin (VIII) from XV.—One gram of XV, treated as described in the preparation of β-amino-*o*-hydroxyacetophenone hydrochloride, yielded 0.6 g. of a crude hydrochloride. This was converted to 3-amino-4-hydroxycoumarin N-acetate, m. p. 222–224°, and to 3,4-diazocoumarin oxide (IX), m. p. 156–157°

Summary

1. 4-Hydroxycoumarin exists completely as the enol.

2. Bromination, nitration, sulfonation and coupling with diazotized amines have been carried out with 4-hydroxycoumarin. These reactions yield 3-substituted-4-hydroxycoumarin.

3. 3-Nitro-4-hydroxycoumarin has been converted to 3-amino-4-hydroxycoumarin, β-amino-*o*-hydroxyacetophenone and β-nitro-*o*-hydroxyacetophenone.

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Studies on 4-Hydroxycoumarin. VIII. Phenylhydrazine Degradation of 3,3′-Methylenebis-(4-hydroxycoumarin)[1]

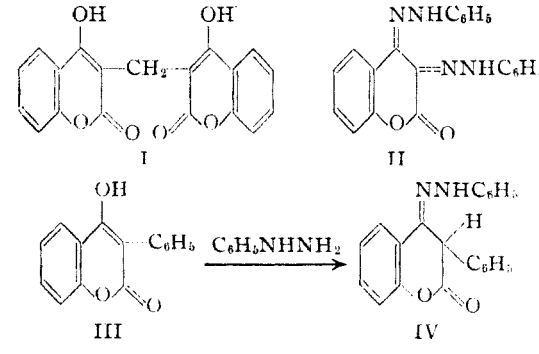
BY CHARLES F. HUEBNER WITH KARL PAUL LINK

A reaction of great importance in the identification of 3,3′-methylenebis-(4-hydroxycoumarin) (I), the anticoagulant in spoiled sweet clover hay,[2] was the cleavage induced by heating with phenylhydrazine at 135°. A red-colored compound (V), $C_{21}H_{16}O_2N_4$, melting at 189–189.5°[3] was obtained. The composition of V suggested that two 4-hydroxycoumarin units were produced by a cleavage of I ($C_{19}H_{12}O_6$) with the loss of the methylene bridge and that by reaction of the nine carbon unit with phenylhydrazine V was produced. This paper deals with the structure of V and offers a rationalization on its formation.

Anschütz[4] reported a red-colored product obtained by heating 4-hydroxycoumarin with phenylhydrazine which melted at 186°. The empirical formula was given as $C_{15}H_{10}O_2N_2$, with a nitrogen content of 11.1%. The nitrogen content of V is 15.8%. Repeating Anschütz's directions, the compound obtained by us was red in color, melted at 189–189.5° and was identical with V as determined by a melting point of a mixture of the two products.

It appears that the Anschütz product and V are identical but that an error was made by Anschütz in the composition and empirical formula of the product. Product V is 1-phenyl-3-(*o*-hydroxyphenyl)-4-benzeneazo-5-pyrazolone or a tautomer thereof. Thus one of the phenylhydrazine residues is enjoined as part of the pyrazolone ring and the other as a benzeneazo group substituted on the pyrazolone ring. Evidence that V is a pyrazolone is indicated by the following.

The aromatic ring in the 4-hydroxycoumarin residue can be excluded as a possible position for the attachment of the phenylhydrazine residues because V gave salicylic acid on fusion with potassium hydroxide. Furthermore, two lines of proof showed that one of the phenylhydrazine residues occupied the 3-position of the 4-hydroxycoumarin residue. When 3-phenyl-4-hydroxycoumarin (III), a compound in which the 3-position is already substituted, was heated with phenylhydrazine at 135° a colorless product containing only one phenylhydrazine residue (IV) was obtained. When 2,3,4-triketochromane-3-phenylhydrazone (II), a compound having a 3-phenylhydrazino substituent, was heated with phenylhydrazine at 135°, V was obtained. When II was refluxed in ethanol with phenylhydrazine acetate (typical conditions for hydrazone formation) V was also formed but in smaller yields. In addition to V an orange product (XI) isomeric with it was produced. XI for reasons given below is assigned the structure 1-phenyl-4-benzeneazo - 5 - (*o* - hydroxyphenyl) - 3 - pyrazolone. To exclude the possibility that the 3-phenylhydrazino group of II might be removed under the conditions of this reaction and give a 4-hydroxycoumarin fragment which would then react with phenylhydrazine to yield V and XI, a 2,3,4-triketochromane-3-(*p*-substituted phenylhydrazone) was treated with phenylhydrazine to yield analogs of V and XI which still retained the *p*-substituent. The *p*-substituents used were methyl and nitro groups.[5]



The presence of enolic hydroxyl groups in V and XI was shown by their solubility in alkali (0.5%). On acidification the parent products were regenerated. V dissolved slowly in the alkali and only by heating the solution. In contrast XI

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station and supported since July, 1940, through special grants from the Graduate Research Committee, Office of Dean E. B. Fred, and the Wisconsin Alumni Research Foundation.

(2) Campbell and Link, *J. Biol. Chem.*, **138**, 21 (1941).

(3) Stahmann, Huebner and Link, *ibid.*, **138**, 513 (1941).

(4) Anschütz, *Ann.*, **367**, 169 (1909).

(5) Huebner and Link, THIS JOURNAL, **67**, 99 (1945).